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Butanol production from wheat straw by simultaneous saccharification and fermentation using *Clostridium beijerinckii*: Part I—Batch fermentation

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ABSTRACT

Five different processes were investigated to produce acetone–butanol–ethanol (ABE) from wheat straw (WS) by *Clostridium beijerinckii* P260. The five processes were fermentation of pretreated WS (Process I), separate hydrolysis and fermentation of WS to ABE without removing sediments (Process II), simultaneous hydrolysis and fermentation of WS without agitation (Process III), simultaneous hydrolysis and fermentation with additional sugar supplementation (Process IV), and simultaneous hydrolysis and fermentation with agitation by gas stripping (Process V). During the five processes, 9.36, 13.12, 11.93, 17.92, and 21.42 $g L^{-1}$ ABE was produced, respectively. Processes I–V resulted in productivities of 0.19, 0.14, 0.27, 0.19, and 0.31 $g L^{-1} h^{-1}$, respectively. It should be noted that Process V resulted in the highest productivity (0.31 $g L^{-1} h^{-1}$). In the control experiment (using glucose), an ABE productivity of 0.30 $g L^{-1} h^{-1}$ was achieved. These results suggest that simultaneous hydrolysis of WS to sugars and fermentation to butanol/ABE is an attractive option as compared with more expensive glucose to ABE fermentation. Further development of enzymes for WS hydrolysis with optimum characteristics similar to fermentation would make conversion of WS to butanol/ABE even more attractive.

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1. Introduction

In response to rising gasoline prices, constant conflicts in the oil-supply region of the world, and depletion of fossil fuels, research and commercialization activities directed toward production of renewable fuels and chemicals such as ethanol and butanol have increased. Renewable resources including molasses, corn, whey permeate, wheat straw (WS), corn stover, corn fiber, and other agricultural byproducts have been promoted as potential feedstocks for production of fuels and chemicals. In 2006, $18.6 \times 10^6 m^3$ (4.86 billion gallons) of ethanol was produced from corn in the United States for use as a liquid fuel [1]. This amount of ethanol was about 3% of gasoline that was used in the United States as transport fuel ($538 \times 10^6 m^3$ or 140 billion gallons) in 2006. In the United States, it has been proposed to replace 30% of gasoline by 2030 using renewable resources as substrates. Estimates suggest that up to 15% of gasoline could be replaced by ethanol using corn as a substrate. Further increase in ethanol production will require the use of biomass (WS, rice straw, switch grass, and other agricultural biomass) as substrates.

Butanol is a superior fuel to ethanol [2] and an industrial solvent that can be produced from renewable resources (mentioned above) employing a number of organisms including

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Clostridium acetobutylicum [3,4] and/or *Clostridium beijerinckii* [5]. Historically, butanol (or acetone–butanol–ethanol (ABE): in the fermentation broth, the typical ratio of ABE is 3:6:1, where butanol is a major product) fermentation is second to ethanol and there were plants that operated during WW I and WW II [6]. However, butanol fermentation could not compete with petrochemically derived butanol due to development of petrochemical industries. The last biobased butanol plant ceased operations in the early 1980s in South Africa due to shortage of molasses, the feedstock for this fermentation, brought on by drought. More recently, research continued to revive this process at the cutting edge of technology. Research has been directed at developing superior microbial cultures [7,8] and process technologies [4,9–12]. As a result of these developments, Dupont (United States) and British Petroleum (UK) have announced plans to commercialize butanol production from biobased substrates [13,14].

In a number of studies [15–20], it has been suggested that cost of substrate is a major factor that influences the production cost of biofuels. Hence, economically viable substrates such as WS, rice straw, and switch grass should be used to produce these biofuels. Unfortunately, neither commercial ethanol-producing cultures nor butanol-producing cultures can hydrolyze these substrates [11,21]. Hence, they need to be hydrolyzed prior to fermentation using a combination of pretreatment (acid, alkali, or ammonia explosion) and hydrolysis (enzymes: cellulase, β-glucosidase, and xylanase) techniques. It should be noted that in contrast to ethanol production by yeasts, hexose and pentose sugars obtained as a result of pretreatment and hydrolysis of these residues can be used by butanol-producing cultures [22]. Pretreatment and hydrolysis are generally performed in two separate reactors due to different and/or adverse conditions. Following pretreatment and hydrolysis, fermentation is carried out. Since WS is an economic substrate, our objective was to combine the hydrolysis and fermentation process (and recovery) in a single step to economize the production of butanol from this substrate. WS has been used to produce butanol in combination with alkali pretreatment and hydrolysis with cellulases [23]. Our aim was to use dilute sulfuric acid pretreatment and three hydrolytic enzymes (cellulase, β-glucosidase, and xylanase) for efficient hydrolysis and saccharification of WS to simple sugars. These studies are considered novel as we combined efficient hydrolysis, fermentation, and ABE recovery technologies in a single process. Use of dilute sulfuric acid is advantageous in the hydrolysis of cellulosic biomass as it hydrolyzes much of the hemicellulose to pentose sugars. The residual cellulose and hemicellulose was hydrolyzed by enzymes. Application of alkali solublizes hemicellulose but does not hydrolyze it [24,25].

2. Materials and methods

2.1. Strain and inoculum development

C. beijerinckii P260 was a generous gift from Professor David Jones, University of Otago, Dunedin, New Zealand. Spores of *C. beijerinckii* were maintained in distilled water at 4 °C. The spores (0.1 mL) were heat shocked at 75 °C for 2 min and transferred to cooked meat medium (CMM; Difco Laboratories, Detroit, MI, USA) for spore germination [26]. In order to prepare liquid CMM, 2.5 g of solid CMM pellets and 0.2–0.4 g glucose were suspended in 20 mL distilled water in a 25 mL screw-capped Pyrex™ bottle. The mixture was autoclaved at 121 °C for 15 min followed by cooling to 35 °C. The heat-shocked spores were incubated at 35 °C for 16–18 h when it was ready for inoculum development.

Six milliliters of actively growing cells (from liquid CMM) was inoculated into 100 mL of inoculum development P2 medium, prepared in a 125 mL screw-capped bottle. The inoculum development P2 medium contained glucose (30 g L^{-1}; Sigma Chemicals, St. Louis, MO, USA), yeast extract (1 g L^{-1}; Sigma Chemicals), and stock solutions (mineral, buffer, and vitamin). The details of the stock solutions have been published elsewhere [27]. The solution containing glucose and yeast extract was sterilized at 121 °C for 15 min followed by cooling to room temperature. At that time, 1 mL of each of the filter sterilized stock solutions was added to 100 mL glucose–yeast extract solution. The culture (inoculum) was allowed to grow for 16–18 h at 35 °C when it was ready to be inoculated into the ABE production medium.

2.2. Wheat straw (WS) pretreatment and separate hydrolysis

WS, obtained from a local farmer, was pretreated and hydrolyzed as described previously [28,29].

2.3. Fermentation

Fermentation studies were conducted either in 250 mL Pyrex™ screw-capped bottles containing 100 mL medium or in a 2.5 L New Brunswick bioreactor (Bioflo-2000, New Brunswick, NJ, USA) containing 1 L medium. Five different hydrolysis, fermentation, and recovery experiments (called Processes I–V) were conducted under different conditions. For Process I, pretreated WS with dilute sulfuric acid (10 mL H_2SO_4 in 990 mL distilled water; no enzyme treatment) was fermented in a 250 mL bottle (100 mL medium) after removing sediments by centrifugation. This process was called pretreatment alone. After removing sediments, the pH of the clear solution was brought to 6.5 using 10 M NaOH. To this solution, 2.5 mL of 40 g L^{-1} sterilized yeast extract (Becton-Dickinson & Co., Sparks, MD, USA) solution was added. Prior to inoculation, 1 mL each of P2 stock solutions was added. At this stage, the bottles were transferred to an anaerobic jar (BBL GasPak™, Sparks, MD, USA) for 48 h for developing anaerobic conditions inside the medium using BD GasPak™ EZ (Sigma Chemicals, St. Louis, MO, USA) envelopes with indicators. Then the bottles were inoculated with 6 mL of actively growing 16–18 h old culture developed above. During fermentation, 1.5 mL samples were taken for sugar and ABE measurement. The samples were centrifuged at 15,000*g* for 3 min to separate sediments, and the clear liquid was stored at −18 °C until it was analyzed for ABE and sugars. Fermentation was conducted at 35 °C until the culture ceased ABE production.

In Process II, WS was pretreated with dilute acid and hydrolyzed separately with enzymes [28]. Upon hydrolysis,

Table 1 – Experimental conditions that were applied to the five processes for butanol production from WS using *C. beijerinckii*

Processes	Conditions					
	Dilute acid (pretreatment)	Sediments	SHF	SSF	Sugar supplementation	Gas stripping for agitation and ABE recovery
I	+	−	+	−	−	−
II	+	+	+	−	−	−
III	+	+	−	+	−	−
IV	+	+	+	−	+	−
V	+	+	−	+	−	+

+, Condition was applied or was present; −, condition was not applied or was not present; SHF, separate hydrolysis and fermentation (hydrolysis performed at 45 °C). Fermentation was initiated when hydrolysis was complete; SSF, simultaneous saccharification and fermentation (hydrolysis performed during fermentation at 35 °C and enzymes were added at the time of inoculation).

the pH of the mixture was brought to 6.5. Yeast extract and P2 solutions were also added at the same level followed by developing anaerobic conditions and inoculation with 6 mL culture. These studies were also carried out in 250 mL screw-capped bottles with 100 mL medium. This process was called separate hydrolysis and fermentation (SHF). These studies also differed from the above (Process I) as sediments were not removed from the wheat straw hydrolysate (WSH). Fermentation was conducted as described above.

In Process III, WS (86 g in 1 L dilute sulfuric acid) was pretreated in a 2 L beaker covered with aluminum foil. Upon cooling the mixture, the pH was adjusted to 6.5 with 10 M NaOH followed by transferring the mixture to the 2.5 L bioreactor (Bioflo-2000). To the bioreactor, 10 mL of autoclaved yeast extract solution (1 g yeast extract dissolved in 10 mL) and 10 mL each of the P2 stock solutions were added. Anaerobic condition in the fermenter was developed by sparging oxygen-free nitrogen gas through the medium for 48 h. During sparging, the mixture was agitated at 150 rpm. After 48 h of sparging with nitrogen gas, the reactor was inoculated with 60 mL of actively growing culture and 6 mL of each of the three enzyme solutions was added. During fermentation, the culture was not agitated mechanically as the culture is negatively affected by mechanical/axial agitation [30]. This process was called simultaneous hydrolysis and fermentation (SSF).

For Process IV, 8.6 g of WS was pretreated with 100 mL dilute sulfuric acid (10 mL per 990 mL water) as described above. Following pretreatment at 121 °C for 1 h, the mixture was cooled to room temperature and its pH was adjusted to 5.0 with 10 M NaOH. Then the enzymes were added and the mixture was incubated at 45 °C for 72 h. Upon hydrolysis, the bottle was kept in an anaerobic jar for 48 h for anaerobiosis. This was followed by adding 6 mL of 400 g L^{-1} presterilized glucose and 2.5 mL of 40 g L^{-1} presterilized yeast extract solutions. After this, P2 medium stock solutions were added prior to inoculation.

For the experiment with agitation employing gas stripping (Process V), 1 L WS medium was prepared in a 2.5 L bioreactor as described for Process III. Fermentation gases (CO_2 and H_2) were recycled after 23 h of fermentation to agitate the culture. Gas stripping is also advantageous by removing ABE from the fermentation broth because butanol is toxic to the culture. A schematic diagram of gas stripping has been given elsewhere [30]. Condensed ABE was recovered from the reactor regularly during the experiment. Table 1 shows all the treatments that were applied to these studies.

2.4. *Analyses*

Fermentation products (ABE, acetic acid, and butyric acid) were analyzed by gas chromatography (GC; 6890N; Agilent Technologies, Wilmington, DE, USA) as described previously [30,31]. Before injection into the GC, the samples were diluted 4-fold with distilled water. The GC was equipped with an autosampler and an integrator. Sugars were measured using Surveyor HPLC (high-performance liquid chromatograph) equipped with an automatic sampler/injector (Thermo Electron Corporation, West Palm Beach, FL, USA). The HPLC column (HPX-87P; Aminex Resin based) was obtained from BioRad (Hercules, CA, USA). Solvent (milliQ water) flow rate was maintained at 0.6 mL min^{-1}. For sugar analysis, the mixture was centrifuged at 15,000*g* for 15 min followed by diluting 20 times and injecting into the HPLC. ABE productivity was calculated as ABE produced in g L^{-1} divided by the fermentation time and is expressed as g L^{-1} h^{-1}. ABE yield was calculated as the total ABE produced divided by the total sugar utilized. Where applicable, cell concentration was measured by an optical density (wavelength 540 nm) method using a previously determined standard curve for *C. beijerinckii* P260 and is expressed as dry weight in g L^{-1} fermentation broth. The results presented here are an average of two replications.

3. Results and discussion

In order to compare results obtained in these studies, a batch fermentation was run in which glucose was used as a substrate. During 72 h of fermentation, the culture produced 21.37 g L^{-1} total ABE, resulting in an ABE productivity of 0.30 g L^{-1} h^{-1}. During the fermentation, 59.4 g L^{-1} glucose was used, resulting in an ABE yield of 0.36 [22].

Following this control experiment, pretreated WS was fermented (Process I). The culture produced 2.72 g L^{-1} acetone, 6.05 g L^{-1} butanol, and 0.59 g L^{-1} ethanol, resulting in a total ABE production of 9.36 g L^{-1} in 50 h. The amount of acids produced was 1.85 g L^{-1} (acetic acid 1.25 g L^{-1} and butyric acid 0.60 g L^{-1}). In the beginning of the fermentation, 2.8 g L^{-1} glucose, 17.8 g L^{-1} xylose, 3.1 g L^{-1} arabinose, and 1.7 g L^{-1} galactose were present. The concentration of mannose was 0 g L^{-1}. During the fermentation, all the sugars (total 25.4 g L^{-1}) were consumed by the culture.

In experiments using Process II, pretreated WS was hydrolyzed using enzymes for 72 h. In order to study whether sediments present in the hydrolysate would inhibit fermentation or contribute to mass transfer inhibition, the hydrolysate was not centrifuged to remove suspended solids. The culture produced 4.52 g L^{-1} acetone, 8.09 g L^{-1} butanol, and 0.77 g L^{-1} ethanol, resulting in a total ABE of 13.38 g L^{-1}. The total ABE production was 43% higher than the above experiment (Process I) run with pretreated WS. The fermentation ceased as all the sugars were used by the culture except 0.5 g L^{-1} galactose. In the beginning of the fermentation, 19.1 g L^{-1} glucose, 17.1 g L^{-1} xylose, 2.6 g L^{-1} arabinose, 0 g L^{-1} mannose, and 3.1 g L^{-1} galactose were present. The total amount of sugar that was initially present in the medium was 41.9 g L^{-1}. During the fermentation, 2.04 g L^{-1} total acids (acetic acid 0.92 and butyric acid 1.12 g L^{-1}) was produced. It appears that there was no inhibition due to presence of sediments as fermentation stopped due to lack of sugars (also see Process IV later).

In Process III, pretreated WS was hydrolyzed simultaneously with fermentation as the enzymes were added to the reactor at the time of inoculation. Visible gas production by the culture was evident within a few hours after inoculation. It took 45 h to complete the fermentation. At the end of fermentation, 3.74 g L^{-1} acetone, 7.40 g L^{-1} butanol, 0.79 g L^{-1} ethanol, 1.12 g L^{-1} acetic acid, and 0.82 g L^{-1} butyric acid were measured. The initial amount of sugars present in the system were 5.2 g L^{-1} glucose, 16.8 g L^{-1} xylose, 2.3 g L^{-1} arabinose, 1.3 g L^{-1} galactose, and 0 g L^{-1} mannose. The amount of residual sugars was 0 g L^{-1}, suggesting that fermentation stopped due to lack of sugars. The culture produced 11.93 g L^{-1} total ABE. This amount of ABE is 27% higher than that obtained in the pretreated WS fermentation (Process I), suggesting that SSF occurred. It should be noted that the optimum conditions of fermentation (temperature 35 °C, pH 6.5–5.0, no agitation) were different from the optimum conditions for hydrolysis (temperature 45 °C, pH 5.0, agitation 80 rpm) using hydrolytic enzymes.

The culture produced less ABE than in the previous experiment (Process II), suggesting that complete hydrolysis did not occur. Perhaps due to different hydrolysis and fermentation conditions the enzymes did not hydrolyze WS completely. In order to investigate whether the hydrolysis was incomplete, the fermentation broth was agitated at 80 rpm, and pH and temperature were adjusted to 5.0 and 45 °C, respectively, for further hydrolysis for 72 h. At the end of saccharification, a sample was taken to measure sugars and ABE. In the broth, 10.5 g L^{-1} glucose and 2.8 g L^{-1} xylose were measured (a total of 13.3 g L^{-1} sugars; each of arabinose, galactose, and mannose were 0 g L^{-1}), thus suggesting that during SSF, 76% hydrolysis was complete. After the second hydrolysis, the concentration of ABE was the same as reported in the above paragraph. While simultaneous saccharification and fermentation was partially successful, it is suggested that development of hydrolytic enzymes with an optimum temperature of 35 °C and a pH 5.0–6.5 could enhance the rate of hydrolysis of WS for SSF.

In the above experiments, ABE levels were lower than usually reported in our previous work [22], possibly due to low sugar levels in the hydrolysate. In order to check whether *C. beijerinckii* P260 would produce a higher level of ABE in the presence of sediments, an experiment was run in which WSH was supplemented with additional sugar (Process IV). The fermentation was run in a batch mode for 96 h and during this time 17.92 g L^{-1} ABE was produced, suggesting that there was no inhibition due to the presence of sediments. The initial sugar level was 51.7 g L^{-1} (glucose 36.2, xylose 12.9, arabinose 1.7, galactose 0.9, and mannose 0 g L^{-1}). The final sugar level was 6.7 g L^{-1} (glucose 2.6 and xylose 4.1 g L^{-1}). *C. beijerinckii* produces 18–25 g L^{-1} ABE using simple sugars [22,28].

In our Process III experiment (without agitation), WS was simultaneously incompletely hydrolyzed (approximately 76%). Less than 100% hydrolysis may have been due to lack of agitation of the mixture and it is likely that enzymes were not mixed well in the reaction mixture. Since mechanical/axial agitation affects butanol-producing cultures adversely [30], agitation by gas stripping was considered as an alternative agitation technique suitable for this work. Previous work demonstrated that agitation caused by gas stripping does not affect butanol-producing cultures adversely [3,22,30]. Additionally, gas stripping removes toxic butanol from the fermentation broth. Hence, during the next experiment (Process V), agitation by gas stripping was provided. In this run, enzymes were added to the reactor at the time of inoculation. The resulting fermentation was vigorous and after 23 h, 9.93 g L^{-1} ABE (acetone 3.61, butanol 5.71, and ethanol 0.61 g L^{-1}) and 1.96 g L^{-1} acids (acetic acid 1.13 and butyric acid 0.83 g L^{-1}) were present in the reaction mixture. The fermentation profile of this experiment is shown in Fig. 1. The gas stripping was helpful both for providing agitation and for removing ABE from the reaction mixture in this experiment (SSF). The total amount of sugar that was present in the fermentation mixture at 23, 29, 47, 54, and 71 h was 0, 0, 0.81, 0.41, and 0.72 g L^{-1}, respectively. This run ended after 71 h of fermentation. From this run, condensed ABE was removed at 29, 47, 54, and 71 h. The individual concentrations of ABE in the condensate are shown in Fig. 2. As the fermentation reached near completion (71 h), the ABE concentration in the recovered stream decreased. At 29 h, the ABE level in the condensed stream was 67 g L^{-1} (acetone 20.4, butanol 45.5, and ethanol 1.1 g L^{-1}) (Fig. 2). At 71 h, when the fermentation was complete, 17.8 g L^{-1} ABE was present in the condensate. The decreased concentration of ABE in the condensate was due to decreased concentration of ABE in the broth.

During the fermentation (Process V), sugar concentrations ranged from 0.0 g L^{-1} at 23 h to 0.7 g L^{-1} at 71 h. At 0 time, a sugar concentration of 26.1 g L^{-1} was present in the reactor. The individual sugar levels are shown in Table 2. From the table it is clear that the culture used sugar faster than it was

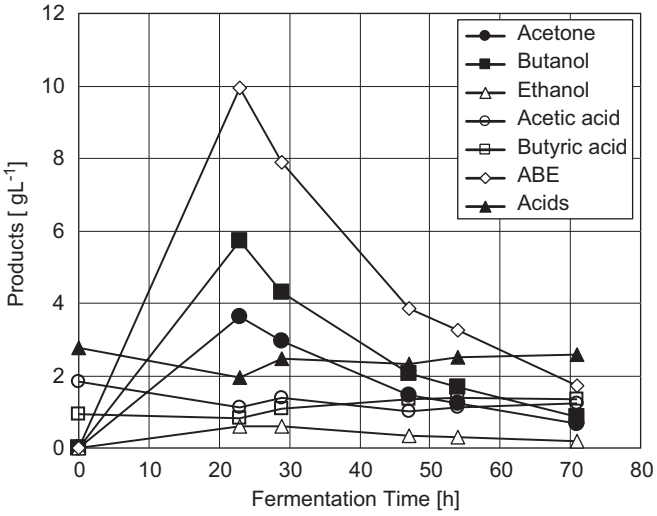

Fig. 1 – Simultaneous hydrolysis and fermentation of wheat straw using *C. beijerinckii* P260 with agitation by gas stripping (Process V). Gas stripping was started at 23 h.

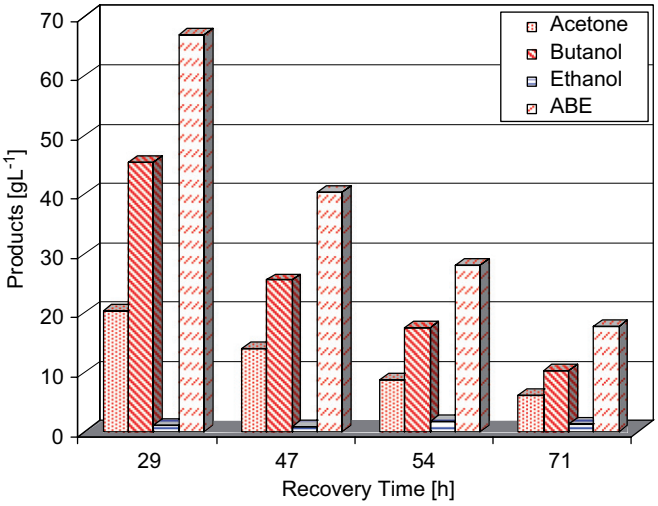

Fig. 2 – ABE in recovered stream from simultaneous hydrolysis and fermentation of WS (Process V).

Table 2 – Concentrations of various sugars present during simultaneous hydrolysis of WS and fermentation to butanol using *C. beijerinckii* (Process V)

Fermentation time (h)	Sugars (gL^{-1})				
	Glucose	Xylose	Galactose	Arabinose	Mannose
00	3.1	17.3	0.9	3.1	1.7
23	0.0	0.0	0.0	0.0	0.0
29	0.0	0.0	0.0	0.0	0.0
47	0.2	0.2	0.4	0.0	0.0
54	0.0	0.0	0.4	0.0	0.0
71	0.0	0.0	0.7	0.0	0.0

released by hydrolysis of the WS (initially). Between 47 and 71 h, 0.8–0.7 gL^{-1} sugars were present (primarily galactose).

After the fermentation was over (Process V), the pH of the reaction mixture was reduced to 5.30 and the temperature was raised to 45 °C to check whether unhydrolyzed cellulosic polymer was present in the system. Agitation was provided at 80–100 rpm. The hydrolysis was carried out for an additional 72 h when a sugar concentration of

Table 3 – Concentrations of various sugars after second hydrolysis (post fermentation—Process V)

Hydrolysis time (h)	Sugars ($g L^{-1}$)				
	Glucose	Xylose	Galactose	Arabinose	Mannose
00	0.0	0.0	0.7	0.0	0.0
24	0.6	0.2	0.7	0.0	0.0
50	0.6	0.3	0.7	0.0	0.0
72	1.4	0.5	0.7	0.0	0.0

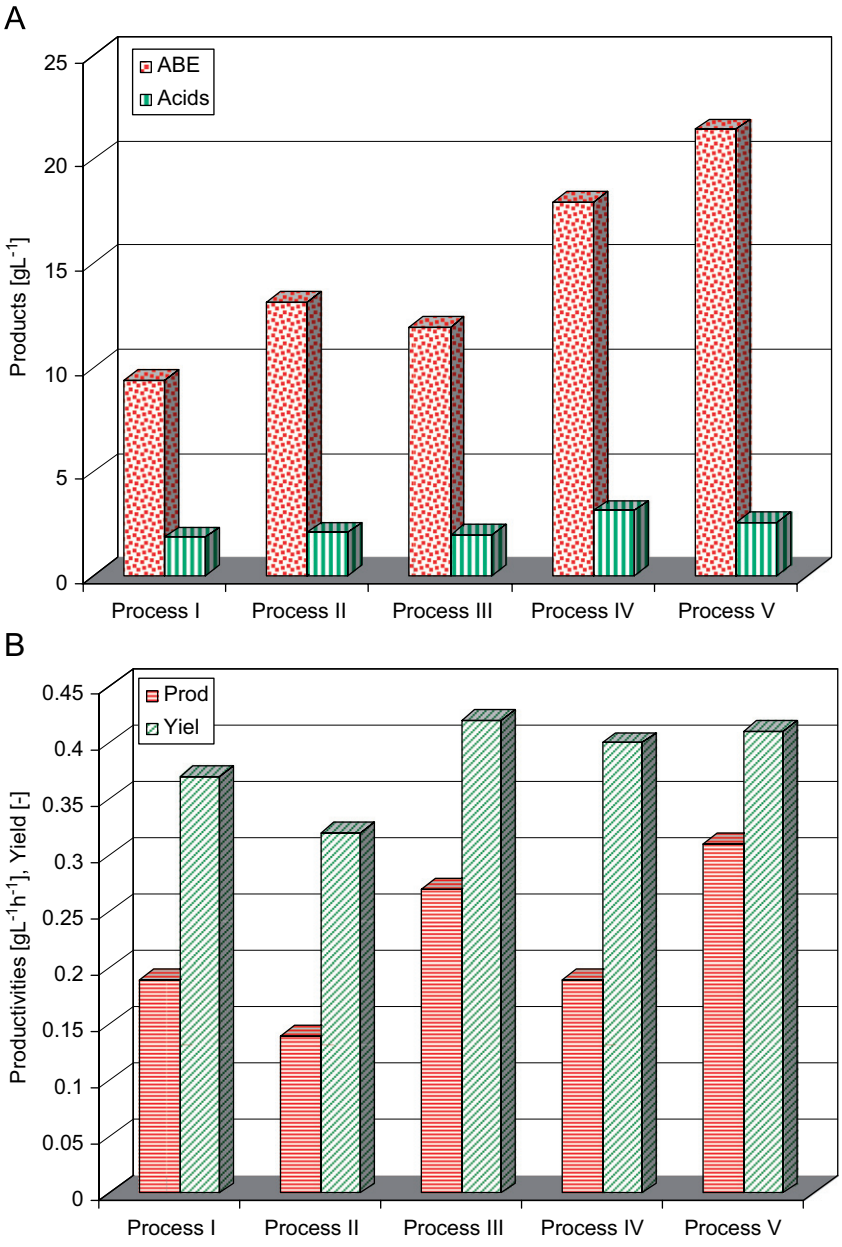

Fig. 3 – Kinetic parameters of butanol production in processes I–V. (A) ABE and acids and (B) productivities and yield.

$2.6\,g L^{-1}$ was measured in the broth. The total sugar concentrations at 24 and 50 h were 0.71 and $1.52\,g L^{-1}$, respectively. The gas stripping experiment suggested that agitation caused by gas stripping assisted in achieving more hydrolysis. During this process, 95% hydrolysis was complete (of "potentially" available sugars using the enzymes employed in these experiments). The various sugar levels are shown in Table 3.

Although there was an increased hydrolysis (Process V), the culture was often deficient in sugars. However, a productivity of 0.31 $g L^{-1} h^{-1}$ was observed. In order to increase productivity, a sugar solution could be fed to the reactor to maintain low levels of sugars. This should be done while simultaneously hydrolyzing WS. Fermentation of WS had a number of challenges including transfer of sterilized WS to the reactor, sampling, mass transfer, and agitation. In spite of these problems, simultaneous saccharification, fermentation, and recovery appear to be an attractive option for butanol/ABE production from WS.

The amounts of ABE and acids are compared for the five processes in Fig. 3A. In these processes (I–V), 1.85, 2.08, 1.94, 3.13, and 2.58 $g L^{-1}$ acids were measured, respectively. The five processes were also compared in terms of productivity and yield (Fig. 3B). Process I resulted in a productivity of 0.19 $g L^{-1} h^{-1}$ and a yield of 0.37. Process II resulted in a productivity of 0.14 $g L^{-1} h^{-1}$ and a yield of 0.32. It should be noted that Process V (simultaneous saccharification and fermentation with agitation by gas stripping) resulted in the highest productivity (0.31 $g L^{-1} h^{-1}$) and a high yield (0.41). In the control experiment using glucose as a substrate, a productivity of 0.30 $g L^{-1} h^{-1}$ and a yield of 0.36 were obtained. The improvement in productivity using WS as a substrate has been achieved by applying different experimental conditions. This suggests that conversion of WS to butanol or ABE is a technically feasible option. Further development of enzymes with optimum conditions that are used in fermentation would make conversion of WS to butanol/ABE more attractive. It should be noted that these results are superior to those reported by Marchal et al. [23]. This increase in yield and productivity is due to the use of appropriate pretreatment (dilute H_2SO_4), hydrolysis (used all the necessary enzymes; cellulase, β-glucosidase, and xylanase), fermentation, and recovery technologies. In these studies, we have been able to hydrolyze >90% of WS. In other studies [23], no account of the extent of hydrolysis has been reported. Further, we are investigating improving hydrolysis efficiency to 100% and increase productivity in an integrated hydrolysis, fermentation, and recovery system.

4. Conclusions

In conclusion, five processes were investigated to produce ABE from WS by *C. beijerinckii* P260. The five processes were fermentation of pretreated WS (Process I), separate fermentation of pretreated and hydrolyzed WS to ABE without removing sediments (Process II), SSF without agitation (Process III), SSF with additional sugar supplementation (Process IV), and SSF with agitation by gas stripping (Process V). During the five processes, 9.36, 13.12, 11.93, 17.92, and 21.42 $g L^{-1}$ ABE was produced, respectively (Fig. 3A). In these processes, 1.85, 2.08, 1.94, 3.13, and 2.58 $g L^{-1}$ acids were measured, respectively. The five processes were also compared in terms of productivity and yield (Fig. 3B). The five processes resulted in productivities of 0.19, 0.14, 0.27, 0.19, and 0.31 $g L^{-1} h^{-1}$, respectively. In these processes, ABE yields of 0.37, 0.32, 0.42, 0.40, and 0.41 have been achieved, respectively. Process V (simultaneous saccharification and fermentation with agitation by gas stripping) resulted in the highest productivity (0.31 $g L^{-1} h^{-1}$) and a high yield (0.41). In the control experiment where glucose was used as a substrate, reactor productivity and an ABE yield of 0.30 $g L^{-1} h^{-1}$ and 0.36 was achieved, respectively. Hence, it is suggested that conversion of WS to butanol or ABE is a technically feasible option. Further development of enzymes with optimum conditions that are used in fermentation would make the conversion of WS to butanol/ABE more attractive. In Process V, the culture was deficient (initially) in sugars, suggesting that sugar utilization was faster than WS hydrolysis. This may have affected the culture negatively, thus reducing reactor productivity. Additionally, hydrolysis of WS was incomplete. For these two reasons, it is recommended that a fed-batch system be used where the culture is fed with additional sugar solution (to avoid deficiency of sugar) when needed. It is also recommended that the fed-batch system be run longer to hydrolyze WS completely. Based on these suggestions a fed-batch system was operated which is described in the next paper [32] of this issue.

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